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13. ABSTRACT (Maximum 200 words) The amount of O <sub>2</sub> available to tissues is essentially the product of cardiac output, [Hb], and O <sub>2</sub> saturation. Saturation depends on PO <sub>2</sub> and the O <sub>2</sub> Hb dissociation curve. With altitude, increased [2,3-DPG] shifts the dissociation curve rightward, but hypocapnia and alkalosis move it leftward. We determined both standard and in vivo P50 in 5 fit subjects decompressed over 42 days in an altitude chamber to the equivalent of the Mt. Everest summit (Operation Everest II). Arterial and venous blood was sampled at five "altitudes" (PB = 760, 429, 347, 282, 253 mmHg), and PO <sub>2</sub> , PCO <sub>2</sub> , pH, O <sub>2</sub> saturation, [Hb] and [2,3-DPG] were measured. As reported previously, 2,3-DPG levels increased from 1.7 (PB = 760) to 3.8 mmol/L (PB = 282). Standard P50 also increased (from 28.2 mmHg at sea level to 33.1 on the summit, p< 0.001). Alone, this would have lowered saturation by 12 percentage points at a summit arterial PO <sub>2</sub> of 30 mmHg. However, in vivo P50 remained between 26 and 27 mmHg throughout due to progressive hypocapnia and alkalosis. Calculations suggest that the increase in standard P50 did not affect summit VO <sub>2</sub> MAX, alveolar, arterial and venous PO <sub>2</sub> 's, but reduced arterial and venous O <sub>2</sub> saturations by 8.4 and 17.4 points, respectively, and increased O <sub>2</sub> extraction by 7.9 percentage points. Reduced saturation was balanced by increased extraction, resulting in no significant overall O <sub>2</sub> transport benefit, thus leaving unanswered the question of the purpose of increased [2,3-DPG] concentrations at altitude.				
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## Hemoglobin P<sub>50</sub> During a Simulated Ascent of Mt. Everest, Operation Everest II

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### ABSTRACT

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**Key Words:** oxygen transport; altitude; exercise; hemoglobin dissociation curve; 2,3-diphosphoglycerate

### INTRODUCTION

**I**N 1985, OPERATION EVEREST II (OE II) was conducted within the USARIEM hypobaric chambers at Natick, Massachusetts (Houston et

al., 1987). In this study, 8 healthy young fit men were gradually decompressed over 42 days from sea level to a pressure equivalent in P<sub>I<sub>O<sub>2</sub></sub></sub> to that on the Everest summit (West, 1983). Blood gas measurements were made at rest and

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during exercise at sea level and at barometric pressures of 429, 347, 282, and 253 mmHg, corresponding to altitudes of approximately 15,000, 20,000, 25,000, and 29,000 ft. Samples were taken from a radial or brachial artery and, except at 15,000 ft, the pulmonary artery as well. From these samples,  $P_{O_2}$ ,  $P_{CO_2}$ , pH,  $O_2$  saturation, Hb concentration, and hematocrit were all measured, along with blood temperature. These data provided the basis for several reports documenting changes in pulmonary gas exchange with simulated ascent, including the effects of exercise (Wagner et al., 1987; Sutton et al., 1988; Cymerman et al., 1989).

What has never been examined from this unique data set is the  $P_{50}$  of hemoglobin (Hb $P_{50}$ ). As original OE II investigators, the authors have reexamined the raw data and determined both standard and in vivo Hb $P_{50}$  of the subjects as a function of simulated altitude. The present paper reports on the methodology for determining Hb $P_{50}$  from such data sets, the effects of the simulated Everest ascent on that variable, and the calculated consequences for maximal  $O_2$  transport and  $\dot{V}_{O_{2MAX}}$ . Based on previous work (Lenfant et al., 1968; Mulhausen et al., 1968; Lenfant et al., 1969; Baumann et al., 1971; Lenfant et al., 1971; Gerlach et al., 1972), it was expected that standard Hb $P_{50}$  would increase with increasing altitude [on the basis of increased levels of 2,3-diphosphoglycerate, which rose from  $1.7 \pm 0.3$  mmol/L at  $P_B = 760$  mmHg to  $3.8 \pm 0.4$  mmol/L at  $P_B = 282$  mmHg (Suter et al., 1988)], but the degree of change and consequences for  $O_2$  saturation of the blood were unpredictable at extreme altitude, especially in the face of counterbalancing effects from progressive hyperventilation and alkalosis.

It was found that standard Hb $P_{50}$  increased substantially and significantly from 28.2 mmHg at sea level to 33.1 mmHg on the summit. This increase would be sufficient to cause a fall of 12 percentage points in  $O_2$  saturation at a  $P_{O_2}$  of 30 mmHg (at constant pH and  $P_{CO_2}$ ). However, because of the progressive hypocapnia and alkalosis during ascent, in vivo  $P_{50}$  remained remarkably constant at about 27 mmHg at all simulated altitudes. Theoretical calculations were performed to assess the importance of the increase in standard Hb $P_{50}$  on diffusive loading of  $O_2$  in the lungs, diffusive

unloading of  $O_2$  in the muscles, and on total  $O_2$  transport. They showed that the increase in  $P_{50}$  would not affect maximal  $O_2$  transport, or alveolar, arterial, and venous  $P_{O_2}$ , but would substantially reduce arterial and venous  $O_2$  saturations and increase fractional muscle  $O_2$  extraction.

## METHODS

### *Timing of measurements in relation to ascent profile*

The ascent profile has been reported previously (Houston et al., 1987) and is somewhat faster than typical field ascents of Everest itself. After sea-level measurements, subjects were decompressed to the equivalent of 15,000 ft (4572 m,  $P_B = 429$  mmHg), and measurements were made at this altitude at days 10 and 11. Subjects then moved to 20,000 ft (6096 m,  $P_B = 347$  mmHg), with measurements over days 18 to 22. They were then taken higher, to 25,000 ft (7620 m,  $P_B = 282$  mmHg) with measurements over days 36 to 40, and on the same day for each subject, further acute decompression to the Everest summit (8840 m,  $P_B = 253$  mmHg) was achieved with a final set of measurements at that time. At every altitude, blood samples were collected at rest and during several incremental steady-state, constant-load exercise levels up to maximal. At all altitudes except 4572 m, samples were collected from both arterial and pulmonary arterial blood. At 4572 m, no pulmonary artery catheter was placed, and so data were available only from the radial artery. Blood samples were placed on ice and locked out to the laboratory adjacent to the chamber where  $P_{O_2}$ ,  $P_{CO_2}$ , pH,  $O_2$  saturation, Hb concentration, and hematocrit were all measured at 37° using standard blood gas electrodes (Radiometer model ABL3) and a co-oximeter (Instrumentation Laboratories model IL 282). Time from collection to measurement was less than 30 min.

### *Calculation of standard Hb $P_{50}$*

Standard  $P_{50}$  is the  $P_{O_2}$  corresponding to a saturation of 50% when the blood temperature is 37°, blood  $P_{CO_2}$  is 40 mmHg, and blood

pH is 7.40. As expected in the present study, no sample during exercise and only resting arterial samples at sea level, reflected these standard conditions. Furthermore, the  $P_{O_2}$ 's of arterial and venous blood were generally far from those corresponding to the actual  $P_{50}$ . Accordingly, the following two methods were devised for determining the standard  $HbP_{50}$ .

*Method 1.* First, for each individual blood sample, Kelman's computer algorithms (Kelman, 1966; Kelman, 1967) were used to calculate the "virtual"  $P_{O_2}$  from the measured value. By this is meant the  $P_{O_2}$  that the actual sample would have shown had temperature been 37°,  $P_{CO_2}$  was 40 mmHg, and pH was 7.40. Then, for each sample, the  $O_2$  saturation was calculated for a trial value of the standard  $HbP_{50}$  using the virtual  $P_{O_2}$ . All samples with  $O_2$  saturations below 15% and above 85% were excluded from the subsequent analysis. This was done to avoid undue influence of errors in data points far from 50% saturation (and where the  $O_2Hb$  dissociation curve becomes less steep or even flat). These exclusion bounds were applied at all altitudes to all subjects without prior inspection of data.

The difference between this calculated saturation and the directly measured  $O_2$  saturation was noted, squared, and summed over all samples included from an individual subject separately at each altitude (except at both 25,000 and 29,000 ft, where blood gas data were combined, because the samples at these two altitudes were taken within 1 h of each other). Then, still for each subject separately, the unique  $HbP_{50}$  was found that produced the best fit (least squares) between the measured and calculated  $O_2$  saturations across the entire set of samples at each altitude.

*Method 2.* A conceptually similar approach was used except that, after using Kelman's algorithms to calculate virtual  $P_{O_2}$  values, the Hill equation was used to compute the best-fit standard  $P_{50}$ . The Hill equation is

$$\text{Saturation} = \frac{P_{O_2}^n}{P_{O_2}^n + P_{50}^n}$$

where  $n$  is the Hill coefficient. After rearranging terms and then taking logarithms, this equation can be expressed as follows:

$$\text{LOG} \frac{\text{saturation}}{(1 - \text{saturation})} = n \times \text{LOG}[P_{O_2}] - n \times \text{LOG}[P_{50}]$$

Using standard linear regression, where  $Y = \text{LOG}[\text{saturation}/(1 - \text{saturation})]$  and  $X = \text{LOG}[P_{O_2}]$ , the values of  $n$  and  $P_{50}$  are readily obtained from the slope and intercept of the regression line between  $Y$  and  $X$ .

#### *Calculation of in vivo $HbP_{50}$ and of the effects of changes in $P_{50}$ on $O_2$ saturation*

Using the standard  $P_{50}$  determined as above, Kelman's algorithms were invoked to determine the  $P_{O_2}$  that would produce 50%  $O_2$  saturation of arterial blood under the actual, measured conditions (i.e., actual temperature,  $P_{CO_2}$ , and pH) at rest for each subject at each altitude.

Hypothetical effects of the changes in standard  $P_{50}$  with altitude on arterial and venous  $O_2$  saturation were then evaluated at the measured blood  $P_{O_2}$  values to assess the physiological importance of  $P_{50}$  at altitude. Again, Kelman's algorithms were used, comparing calculated saturations using the sea-level standard  $P_{50}$  and the summit standard  $P_{50}$ . In these calculations, the actual temperature,  $P_{CO_2}$ , and pH values were utilized.

Finally, using reported numerical analysis methods (Wagner, 1996), the consequences of the changes in  $HbP_{50}$  were examined for diffusive uptake of  $O_2$  in the lungs and diffusive unloading of  $O_2$  in the muscles, for systemic  $O_2$  delivery, and for maximal  $\dot{V}_{O_2}$ . The data needed to conduct these calculations were the same as used in the above analysis (Wagner, 1996) from OE II.

#### *Statistical analysis*

Data are presented as mean  $\pm$  standard error of the mean. Standard error was chosen rather than standard deviation because the interest is in group mean outcomes. With 5 subjects, standard deviation is 2.236 greater than standard error. Repeated measures ANOVA was used to determine the significance of the



changes in  $P_{50}$  with altitude, and a  $p$  value of 0.05 or less was taken to reflect significance.

## RESULTS

Although 8 subjects began the ascent, only 5 provided data at all altitudes. Since the present analysis focuses on changes in  $P_{50}$  from sea level to altitude, it is important to include data from only those subjects who provided sam-

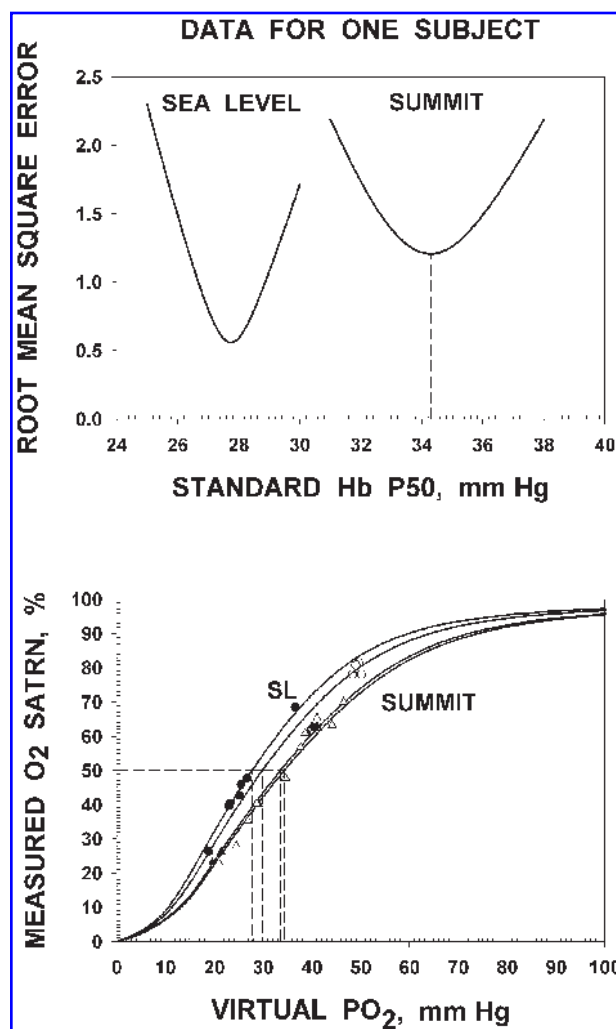


FIG. 1. Top: Identification of standard  $P_{50}$  by the  $P_{O_2}$  associated with minimal root mean square error between measured and calculated  $O_2$  saturations in one subject at two altitudes. Bottom: Data at four altitudes from the same subject as in the top panel, plotted as saturation- $P_{O_2}$  pairs, showing the dissociation curve of best fit at each altitude: sea level, closed circles; 429 mmHg, open circles; 347 mmHg, closed triangles; 282/253 mmHg, open triangles. Dashed lines show  $P_{50}$  increasing from 27.7 to 34.3 mmHg.

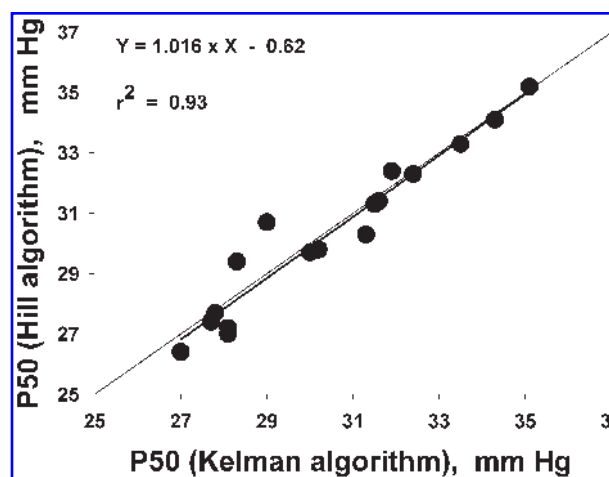


FIG. 2. Agreement ( $r^2 = 0.93$ , data on the line of identity) between the two methods for estimating standard  $P_{50}$ , one using the Hill equation (ordinate) and the other using the Kelman algorithms. Each data point represents one subject at one altitude.

ples at all altitudes. Thus the number of subjects whose data form the basis of this paper is 5.

### Method of determination of standard $HbP_{50}$

The upper panel of Fig. 1 shows the relationship between the sum of squares of the differences between measured and calculated saturations for one representative subject at sea level and at the summit. The ordinate actually expresses the square root of the sum of squares divided by the number of samples used in the calculation and indicates the robust nature of this approach. The lower panel of this figure plots the individual data points for the same subject across all altitudes against  $O_2$ Hb dissociation curves of the corresponding least-squares best-fit  $P_{50}$  values. As the lower panel shows, the potential effects of this rightward shift are substantial. For example, at an arterial  $P_{O_2}$  of 30 mmHg, similar to values measured on the summit (Sutton et al., 1988), a rightward shift of this magnitude would lead to a 12 percentage point reduction in saturation, from ~55% to ~43% in this case. This would in turn reduce arterial  $O_2$  concentration by about 22%.

Figure 2 shows the relationship between standard  $P_{50}$  values determined by the two different methods. While 5 subjects were studied at five altitudes, only 17 points appear in the figure. This is for two separate reasons. First,

at 15,000 ft, in the absence of venous blood samples, data sets from 3 subjects were associated with saturations that differed so little between rest and exercise within each subject that the Hill linear regression method failed to work. Second, the data at 25,000 and 29,000 ft were combined because the blood samples at these two altitudes were taken within about 1 h of each other. The figure shows that the two methods produced the same values, with the regression producing a relationship not different from the identity line and with an  $r^2$  value of 0.93.

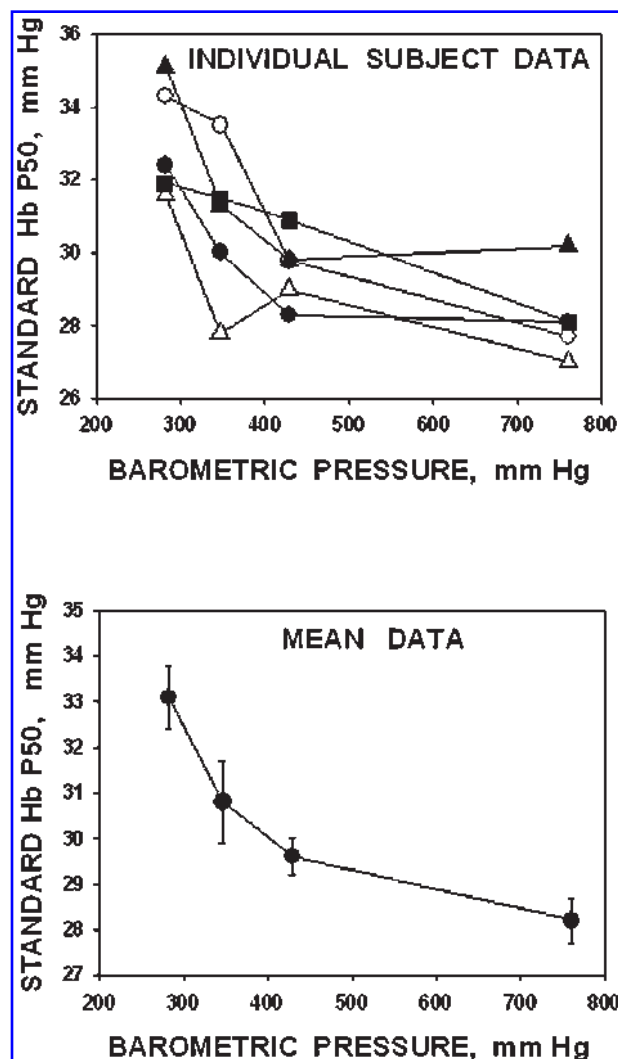


FIG. 3. Standard P<sub>50</sub> increasing with progressive simulated ascent indicated by barometric pressure. Top panel shows individual data; bottom panel group shows mean data, with P<sub>50</sub> increasing from 28.2 to 33.1 mmHg,  $p < 0.001$ .

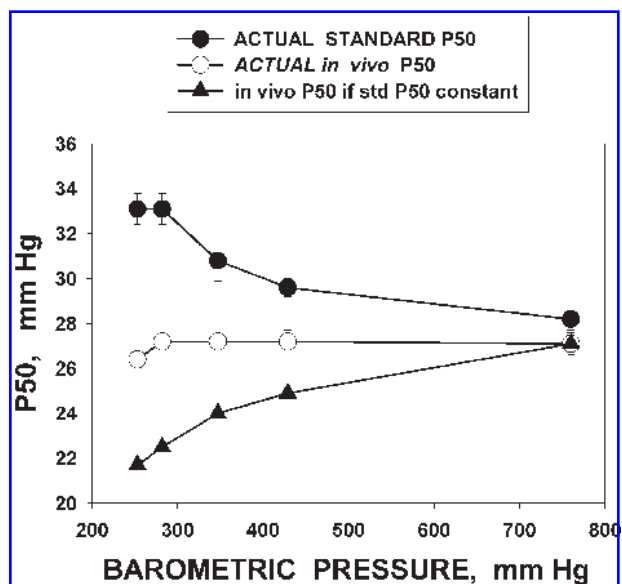


FIG. 4. P<sub>50</sub> changes with altitude: Closed circles show standard P<sub>50</sub> increasing with ascent; open circles show *in vivo* P<sub>50</sub> that takes into account the progressive alkalosis and hypocapnia with continuing ascent. *In vivo* P<sub>50</sub> is independent of altitude due to the counterbalancing effects of rightward- and leftward-shifting influences. Closed triangles show how *in vivo* P<sub>50</sub> would change if standard P<sub>50</sub> had not increased with ascent.

#### Standard HbP<sub>50</sub> results

Figure 3 shows the standard HbP<sub>50</sub> values (method 1) for all 5 subjects at all altitudes. The upper panel displays individual data and the lower panel the mean data for the 5 subjects. There is a progressive and substantial rise in standard P<sub>50</sub> from 28.2 to 33.1 mmHg as barometric pressure falls from 760 to 282 mmHg ( $F = 16.0$ ,  $df = (3, 12)$ ,  $p < 0.001$ ).

#### *In vivo* HbP<sub>50</sub> results

Figure 4 compares the standard HbP<sub>50</sub> (from Fig. 3, closed circles) to the *in vivo* P<sub>50</sub> values that take into account temperature, P<sub>CO<sub>2</sub></sub> and pH (open circles). *In vivo* P<sub>50</sub> remains independent of altitude at between 26 and 27 mmHg. Also presented in Fig. 4 are hypothetical values for *in vivo* P<sub>50</sub> had the standard P<sub>50</sub> not risen during ascent (closed triangles). There would have been a substantial fall to about 22 mmHg at the highest altitudes caused by the progressive alkalosis and hypocapnia.

Figure 5 shows resting arterial P<sub>CO<sub>2</sub></sub> and pH expressed at body temperature for the 5 sub-

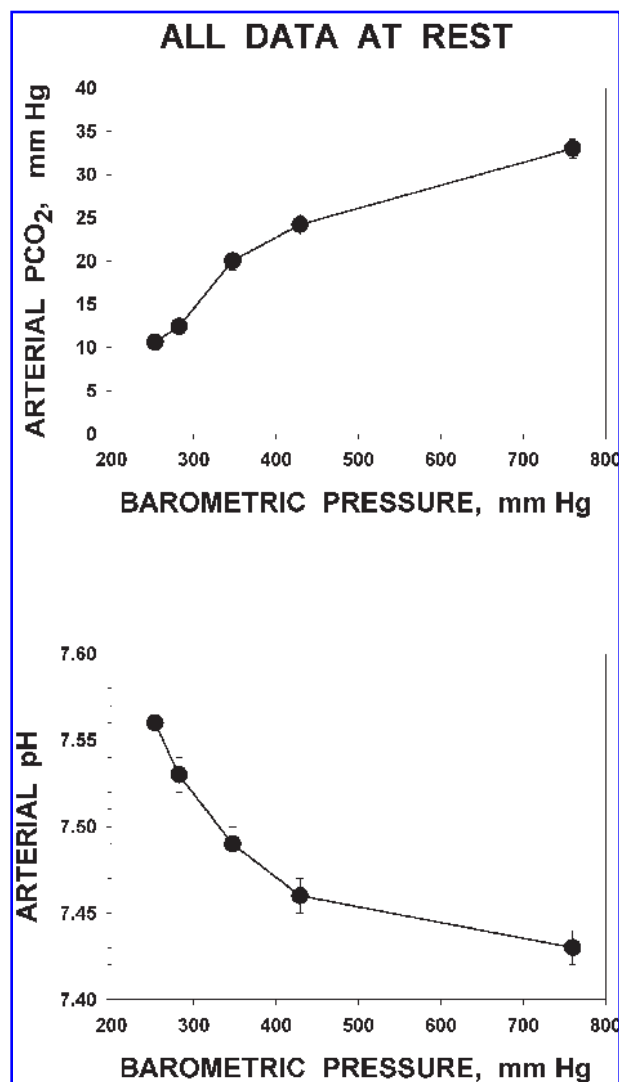


FIG. 5. Progressive fall in arterial  $P_{CO_2}$  and rise in arterial pH with continued ascent underlying the rightward-shifting effects of hyperventilation at altitude.

jects at all altitudes to explain the substantial effect of hyperventilation and alkalosis on in vivo  $P_{50}$ . The changes with altitude are, as expected, highly significant ( $F > 29$ ,  $df = (4, 16)$ ,  $p < 0.0001$ ).

For conditions of maximal exercise on the summit, Table 1 shows the calculated values for alveolar, arterial, and venous blood gas variables under two circumstances: with the actual sea-level standard  $P_{50}$  of 28.2 mmHg and with the actual summit standard  $P_{50}$  of 33.1 mmHg. For these calculations, arterial  $P_{CO_2}$  was taken to be 9 mmHg and base excess to be  $-10$  meq/L. Blood [Hb] was 18.0 g/dL, cardiac output 16 L/min, alveolar ventilation 165

L/min BTPS, lung diffusing capacity 100 mL/(min/mmHg) and muscle diffusing capacity 60 mL/(min/mmHg), all as listed previously (Wagner, 1996).

Several differences and some similarities can be seen. The similarities are in alveolar, arterial, and venous  $O_2$  tensions and in maximal  $\dot{V}_{O_2}$ . The differences are in  $O_2$  saturation of arterial and venous blood, in systemic  $O_2$  delivery, and in muscle  $O_2$  extraction. In essence, lower arterial  $O_2$  saturation is compensated for by greater muscle  $O_2$  extraction.

## DISCUSSION

### *Methodology for determining $P_{50}$*

There are several ways of measuring Hb $P_{50}$ . One involves inscribing the entire  $O_2$  dissociation curve under standard conditions (temperature 37°,  $P_{CO_2}$  40 mmHg, pH 7.40). This was achieved by Imai et al (Imai et al., 1970), but the device was not available during OE II in 1985 when the blood samples were taken. Another method is to mix equal volumes of completely deoxygenated and completely oxygenated blood prepared from a single divided sample from the same subject, keeping both aliquots at standard temperature,  $P_{CO_2}$ , and pH. The  $P_{O_2}$  of the mixed blood is the  $P_{50}$  because the saturation must, by mass conservation, be 50%. This requires tonometry with special gas mixtures to both oxygenate and deoxygenate blood and keep  $P_{CO_2}$  at 40 mmHg and pH at 7.40. It is hard to completely rid blood of all  $O_2$ , and fully oxygenated blood has significant amounts of dissolved  $O_2$  that has to be taken into account. Both of these approaches use single blood samples.

The alternative approach, as used here, is to apply accepted algorithms (Kelman, 1966; Kelman, 1967) that correct  $P_{O_2}$  for temperature,  $P_{CO_2}$  and pH when different from standard conditions. Then, by using all available already-measured blood samples analyzed together, on the hypothesis that on a given day standard  $P_{50}$  is constant between rest and exercise, between arterial and venous blood, and as temperature, pH, and  $P_{CO_2}$  change, a least-squares, best-fit approach can identify

TABLE 1. CALCULATED DIFFERENCES IN O<sub>2</sub> TRANSPORT AND UTILIZATION AT MAXIMAL EXERCISE ON THE SUMMIT AS A FUNCTION OF P<sub>50</sub>

Variable	Standard P <sub>50</sub> (28.2 mmHg)	Standard P <sub>50</sub> (33.1 mmHg)	Ratio
Alveolar PO <sub>2</sub> , mmHg	34.6	34.7	1.00
Arterial PO <sub>2</sub> , mmHg	25.4	25.8	1.02
Venous PO <sub>2</sub> , mmHg	17.9	17.1	0.96
Arterial O <sub>2</sub> saturation, %	64.5	56.1	0.87
Venous O <sub>2</sub> saturation, %	36.9	19.5	0.53
Systemic O <sub>2</sub> delivery, L/min	2.59	2.26	0.87
Systemic O <sub>2</sub> extraction, %	57.1	65.0	1.13
Maximal $\dot{V}O_2$ , L/min	1.48	1.47	0.99

the single P<sub>50</sub> for which the calculated O<sub>2</sub> saturations differ least from the measured values from the same blood samples. By using information from many samples, random error is intrinsically less than in either of the above two approaches, which depend on a single sample. Figure 1 shows the robustness of this method and the reasonableness of the assumptions laid out above. At each altitude, the P<sub>O<sub>2</sub></sub>-saturation pairs do lie closely on a single dissociation curve. The minimum value of the parabolic relationships in the top panel represent the per sample mean difference between calculated and measured saturation. In these two examples, that difference is just 0.5 percentage point of saturation at sea level and 1.1 percentage points at altitude. Thus, this method adds something valuable to the analysis of blood gas measurements in general—an estimate of reproducibility of the integrated electrode plus co-oximeter system.

#### *Why is standard HbP<sub>50</sub> increased with altitude?*

This question is posed at two levels: What is the biochemical basis of the rightward shift, and what is the (presumed) physiological advantage to the organism from a rightward shift on ascent to altitude?

The biochemical basis is most likely the result of an increase in 2,3-diphosphoglycerate [2,3-DPG] levels induced by hypoxia (Chanutin and Curnish, 1967; Lenfant et al., 1969; Boushel et al., 2000). The 2,3-DPG levels were measured at three elevations in OE II: P<sub>B</sub> = 760, 347, and 282 mmHg; and the values were  $1.7 \pm 0.3$ ,  $3.0 \pm 0.2$ , and  $3.8 \pm 0.4$  mmol/L, respectively

(Sutton et al., 1988). Our results therefore add to the already strong evidence that 2,3-DPG levels increase with altitude and are responsible for the increase in standard P<sub>50</sub> (Lenfant et al., 1968; Lenfant et al., 1969; Lenfant et al., 1971). Other than from increased temperature, increased P<sub>CO<sub>2</sub></sub>, or reduced pH, rightward shifting of the O<sub>2</sub> dissociation curve is known to occur only from increased levels of [2,3-DPG]. Because it is standard P<sub>50</sub> that increased in the present study, changes in temperature, P<sub>CO<sub>2</sub></sub>, or pH cannot be invoked as an explanation.

The question of what biological advantage the substantial (5 mmHg) increase in standard P<sub>50</sub> might convey to the organism is more vexing. Conventional arguments state that at altitude a leftward shift enhances diffusive loading of O<sub>2</sub> from alveolar gas into the pulmonary capillaries in the lungs (Bencowitz et al., 1982). This is because during the diffusive process a more hyperbolic, less linear dissociation curve enables large increases in blood O<sub>2</sub> content without large increases in P<sub>O<sub>2</sub></sub>. Maintaining a low blood P<sub>O<sub>2</sub></sub> in turn favors diffusive loading by preserving the gradient in P<sub>O<sub>2</sub></sub> between alveolar gas and the capillaries. Thus a rightward shift would impair O<sub>2</sub> uptake by the lungs.

Similar conventional reasoning suggests that in the skeletal muscles a rightward shift enhances O<sub>2</sub> unloading (West, 1995). Here a similar argument is used in reverse: blood P<sub>O<sub>2</sub></sub> can be kept high, enhancing diffusion to mitochondria, despite reducing saturation (i.e., increasing off-loading) as the curve shifts rightward. The logical dilemma here is that standard P<sub>50</sub> does not oscillate between arterial and venous blood. As a result, what is



“gained” in the muscles is “lost” in the lungs as the dissociation curves shifts rightward (and vice versa should the standard  $P_{50}$  be reduced). Indeed, previous calculations (Wagner, 1997) have shown this counterbalance, and the specific application of these principles to the present situation confirms the futility of a change in standard  $P_{50}$  as a tactic for enhancing overall, integrated  $O_2$  transport through the whole  $O_2$  pathway (lungs, heart and circulation, blood and tissues). In the present situation, the increase in standard  $P_{50}$  has led to lower saturation of arterial and venous blood and greater fractional  $O_2$  extraction by the muscles, such that maximal  $O_2$  transport overall remained unaffected (Table 1).

Perhaps the increase in 2,3-DPG levels on ascent is one of several other possible examples of well-known physiological responses to cellular hypoxia whose primary purpose may be to respond to a different stress. More explicitly, the polycythemia of altitude has most commonly been shown not to materially enhance exercise capacity (Winslow et al., 1981). It would be easier to argue that this erythropoietic hypoxic response evolved over time to stimulate red cell production in response to cellular hypoxia following anemia (nutritional or traumatic) at low altitude, rather than as a response to hypoxia at altitude. Rebuilding red cell mass that has been diminished by nutritional or traumatic factors would seem to be a rational and specific restorative response. The altitude response may therefore be essentially an epiphenomenon. Similar arguments have been made about hypoxic pulmonary vasoconstriction: its primary purpose may be to diminish pulmonary blood flow during fetal development (West, 1995). Its postnatal activity may be again an epiphenomenon. Here this is even easier to imagine because the effect of vasoconstriction is of course pulmonary hypertension, which places a load on the right heart, hardly an advantage to the organism. We already know that the ability of hypoxic vasoconstriction to restore ventilation-perfusion relationships in the lung, another suggested reason for having this response, is far from perfect. Studies comparing  $\dot{V}_A/\dot{Q}$  relationships breathing air and  $O_2$  do show an effect of vasoconstriction that improves these relationships, but

the effect is small, and the fact remains that most patients with lung disease show severe  $\dot{V}_A/\dot{Q}$  inequality despite the presence of hypoxic vasoconstriction (Wagner et al., 1975; Wagner, 1977).

To return to the possible evolutionary role for [2,3-DPG], diffusion in the lung at rest in normoxia is a process with a large amount of excess capacity. Thus, capillary  $P_{O_2}$  equilibrates with that in alveolar gas in just one-third of the available transit time in normal lungs (Wagner and West, 1972). Even during maximal exercise, normal subjects, other than trained athletes, are usually not diffusion limited in the lungs, and even then only about 50% of trained athletes have evident exercise-induced hypoxemia (Dempsey et al., 1984; Powers et al., 1988). In contrast, there is good evidence that muscle  $O_2$  unloading during normoxic exercise is usually limited by muscle  $O_2$  diffusive conductance (Roca et al., 1989), since intracellular  $P_{O_2}$  is far below that of even muscle venous blood. Thus, enhancing muscle  $O_2$  unloading during normoxic exercise by right-shifting the dissociation curve (when anemia develops from nutritional or traumatic causes) may well enhance  $\dot{V}_{O_{2MAX}}$  because there is more diffusive reserve in the lungs to compensate for the negative effects of such a shift on diffusive uptake. It is certainly known that 2,3-DPG levels are increased in anemias (Valeri and Fortier, 1969; Torrance et al., 1970; Lichtman et al., 1971; Boning and Enciso, 1987; Wallis et al., 2005).

#### *Standard versus in vivo $P_{50}$ : Interplay between standard $P_{50}$ , alkalosis, and hypocapnia*

It is curious that the progressive rightward shift we saw in the standard dissociation curve with altitude was precisely counterbalanced at every altitude by an equally progressive leftward shift from increasing alkalosis and hypocapnia, as shown in Fig. 4. That these opposing forces are in play has been known for many years (see Winslow et al., 1981; Mairbaur et al., 1990). Such precise homeostasis usually suggests a regulatory process, which would in this case somehow be mediated by  $O_2$  levels somewhere in the  $O_2$  pathway; but OE II was clearly not designed to investigate whether such is the case or not. Thus, the constancy of

in vivo  $P_{50}$  may be either an active result of some regulatory process, or it may simply be a chance observation with little if any functional significance. Given our discussion of the lack of importance of  $P_{50}$  on maximal  $O_2$  transport at altitude, one is inclined to think the latter, but the present data cannot help decide the issue.

*Comparison with AMREE from sea level to 6300 m*

Winslow et al. (1984) made measurements of  $P_{50}$  during the 1981 American Medical Research Expedition to Everest (AMREE). It appears that when  $P_{50}$  was corrected for pH (that is, corrected from actual pH to 7.40), the corrected values increased by 1.7 mmHg from 28.1 mmHg at sea level to 29.8 mmHg at 6300 m. This is similar to the present findings in which standard  $P_{50}$ , 28.2 mmHg at sea level, increased by 2.6 mmHg to 30.8 mmHg at 6100-m equivalent altitude. Both Winslow's and the present data further suggest that this increase in standard  $P_{50}$  is counterbalanced by progressive hyperventilation and alkalosis so that in vivo  $P_{50}$  remains essentially independent of altitude. Therefore, up to 6300 m, our findings appear essentially similar to those reported by Winslow. This is further supported by the similarity in levels of [2,3-DPG]. Up to 6100 to 6300 m, the highest altitudes for which there are both AMREE and OE II [2,3-DPG] data, there is no difference. Thus, in AMREE, the [2,3-DPG] concentration at sea level was 0.84 mol/mol Hb and at 6300 m it was 1.04 mol/mol Hb (Winslow et al., 1984). Converting to mmol/L blood using Winslow's [Hb] data allows a comparison with OE II values. In these units, the AMREE values become 1.9 and 3.0 mmol/L, respectively. Corresponding values from OE II were 1.7 and 3.0 mmol/L (Sutton et al., 1988).

*Comparison with AMREE above 6300 m*

Above 6300 m, Winslow found that in the one available subject, estimated summit  $P_{50}$  expressed at a pH of 7.4 was only 27.4 mmHg. It is not clear if this estimate is also expressed at a  $P_{CO_2}$  of 40 mmHg or corrects only for pH. Given the estimated summit arterial  $P_{CO_2}$  of 7.5

mmHg, the  $P_{CO_2}$  correction to 40 mmHg (without change in pH) would raise the estimate of  $P_{50}$  by 2.6 mmHg. Furthermore, this subject may be an extreme case, since his  $P_{50}$  expressed at pH 7.4 was more than 4 mmHg lower than that of the only other subject assessed above 6300 m. It is also substantially lower than the group mean data at 6300 m. If these factors are taken into account, it is possible that the average AMREE subject would have had a standard  $P_{50}$  some 6 mmHg higher than 27.4 mmHg, in the region of 33 mmHg, as we found in the present study. Unfortunately, above 6300 m, Winslow's results come from just one (or two) subjects and without arterial blood sampling. Our data come from 5 subjects, each providing multiple arterial and venous samples at all altitudes, and must be considered more reliable and representative.

Winslow also calculated for the single subject that the estimated severe degrees of hypocapnia and alkalosis ( $P_{CO_2}$  7.5 mmHg, pH 7.78) would cause in vivo  $P_{50}$  on the summit to fall substantially, to about 19 mmHg. On the other hand, we found no significant change of in vivo  $P_{50}$  with altitude, even at the summit (Fig. 4). This is because (1) the standard  $P_{50}$  continued to increase in our subjects above 6100 m, and (2) the measured degrees of hypocapnia and alkalosis ( $P_{CO_2}$  10.6 mmHg, pH 7.56) were not as extreme as estimated in AMREE.

The differences between Winslow's and our studies above 6300 m may therefore be explained by several possible factors. First, Winslow's single subject may not have been a typical subject. Second, if correction was made only for pH and not also for  $P_{CO_2}$ , standard  $P_{50}$  would have been underestimated by Winslow. Third, the ascent profiles of AMREE and OE II were different, with OE II subjects "ascending" more quickly than those in AMREE. AMREE subjects appear to have achieved greater  $P_{CO_2}$  reduction and were more alkalotic, which would have together caused a relative leftward shift of the dissociation curve, all other factors equal. This could explain part of the in vivo, but not the standard,  $P_{50}$  differences. However, the difference in in vivo  $P_{50}$  according to whether  $P_{CO_2}$  was 7.5 as in AMREE or 10.6 mmHg as in OE II is only 0.5 mmHg.

*Consequences for exercise at altitude*

This paper confirms through theoretical analysis that the observed changes in P<sub>50</sub> at altitude do not confer increased ability to exercise. It would be difficult, especially in humans, to conduct an interventional study to directly test this hypothesis. Thus the present conclusions await experimental confirmation. However, isolated perfused muscle studies have been conducted wherein standard P<sub>50</sub> was increased (Richardson et al., 1998) or decreased (Hogan et al., 1991), and predictable increases and decreases in maximal  $\dot{V}_{O_2}$  were recorded at constant blood flow and arterial O<sub>2</sub> delivery. These studies in part confirm the present concept of how shifts in the dissociation curve affect O<sub>2</sub> transport and fit directionally with the OE II data presented herein.

## CONCLUSIONS

The principal message would seem to be that, while traditional arguments about how the dissociation curve shifts to the left favor pulmonary O<sub>2</sub> uptake, whereas rightward shifts favor tissue O<sub>2</sub> unloading may be true, any given curve shift will produce counterbalancing effects in the lungs and tissues that lead to little net effect on maximal  $\dot{V}_{O_2}$ , especially at altitude. This is the case in the present situation, where the 5-mmHg increase in standard P<sub>50</sub> found as subjects ascended from sea level to the equivalent of the Everest summit was calculated to reduce arterial and venous O<sub>2</sub> saturations substantially, but at the same time increase muscle O<sub>2</sub> extraction to preserve maximal O<sub>2</sub> transport.

The individual values for arterial and venous P<sub>O<sub>2</sub></sub>, P<sub>CO<sub>2</sub></sub>, pH, O<sub>2</sub> saturation, Hb, and hematocrit are available from the corresponding author by request.

## REFERENCES

- Baumann R., Bauer C., and Bartels H. (1971). Influence of chronic and acute hypoxia on oxygen affinity and red cell 2,3-diphosphoglycerate of rats and guinea pigs. *Respir. Physiol.* 11:135.
- Bencowitz H.Z., Wagner P.D., and West J.B. (1982). Effect of change on P<sub>50</sub> on exercise tolerance at high altitude: a theoretical study. *J. Appl. Physiol.* 53:1487–1495.
- Boning D. and Enciso G. (1987). Hemoglobin-oxygen affinity in anemia. *Blut* 54:361–368.
- Boushel R.C., Calbet J.A.L., Rådegran G., S ndergaard H., Wagner P.D., and Saltin B. (2000). Parasympathetic neural control of heart rate and cardiac output in chronic hypoxia. *Blood Gas News* 9:14–17.
- Chanutin A., and Curnish R.R. (1967). Effect of organic and inorganic phosphates on the oxygen equilibrium of human erythrocytes. *Arch. Biochem.* 121:96–102.
- Cymerman A., Reeves J.T., Sutton J.R., Rock P.B., Groves B.M., Malconian M.K., Young P.M., Wagner P.D., and Houston C.S. (1989). Operation Everest II: maximal oxygen uptake at extreme altitude. *J. Appl. Physiol.* 66:2446–2453.
- Dempsey J.A., Hanson P.G., and Henderson K.S. (1984). Exercise-induced arterial hypoxemia in healthy human subjects at sea level. *J. Physiol. (Lond.)* 355:161–175.
- Gerlach E., Duham J., and Deuticke B. (1972). 2,3-DPG metabolism of red cells: regulation and adaptive changes during hypoxia. In: *Oxygen Affinity of Hemoglobin and Red Cell Acid-Base Status*. M. Rorth and P. Astrup, eds. Munksgaard, Copenhagen; pp. 552–569.
- Hogan M.C., Bebout D.E., and Wagner P.D. (1991). Effect of increased Hb-O<sub>2</sub> affinity on  $\dot{V}_{O_{2max}}$  at constant O<sub>2</sub> delivery in dog muscle *in situ*. *J. Appl. Physiol.* 70:2656–2662.
- Houston C.S., Sutton J.R., Cymerman A., and Reeves J.T. (1987). Operation Everest II: man at extreme altitude. *J. Appl. Physiol.* 63:877–882.
- Imai K., Morimoto H., Kotani M., Watari H., Hirata W., and Kuroda M. (1970). Studies on the function of abnormal hemoglobins. I. An improved method for automatic measurement of the oxygen equilibrium curve of hemoglobin. *Biochim. Biophys. Acta* 200:189–196.
- Kelman, G.R. (1966) Digital Computer Subroutine for conversion of oxygen tension into saturation. *J. Appl. Physiol.* 21:375–376.
- Kelman, G.R. (1967) Digital Computer Procedure for conversion of P<sub>CO<sub>2</sub></sub> into blood CO<sub>2</sub> content. *Resp. Physiol.* 3:111–115.
- Lenfant C., Torrance J., English E., Finch C.A., Reynafarje C., Ramos J., and Faura J. (1968). Effect of altitude on oxygen binding by hemoglobin and on organic phosphate levels. *J. Clin. Invest.* 47:2652–2656.
- Lenfant C., Torrance J.D., and Reynafarje C. (1971). Shift of the O<sub>2</sub>-Hb dissociation curve at altitude: mechanism and effect. *J. Appl. Physiol.* 30:625–631.
- Lenfant C., Ways P., Aucutt C., and Cruz J. (1969). Effect of chronic hypoxic hypoxia on the O<sub>2</sub>-Hb dissociation curve and respiratory gas transport in man. *Respir. Physiol.* 7:7–29.
- Lichtman M.A., Murphy M.S., Whitbeck A.A., and Kearny E.A. (1971). Oxygen binding to haemoglobin in subjects with hypoproliferative anemia, with and without chronic renal disease: role of pH. *Br. J. Haematol.* 27:439–452.

- Mairbaur H., Schobersberger W., Oelz O., Bartsch P., Eckardt K.U., and Bauer C. (1990). Unchanged in vivo P50 at high altitude despite decreased erythrocyte age and elevated 2,3-diphosphoglycerate. *J. Appl. Physiol.* 68:1186–1194.
- Mulhausen R.O., Astrup P., and Mellemgaard K. (1968). Oxygen affinity and acid-base status of human blood during exposure to hypoxia and carbon monoxide. *Scand. J. Clin. Lab. Invest.* 22:9.
- Powers S.K., Dodd S., Lawler J., Landry G., Kirtley M., McKnight T., and Grinton S. (1988). Incidence of exercise induced hypoxemia in elite endurance athletes at sea level. *Eur. J. Appl. Physiol. Occup. Physiol.* 58:298–302.
- Richardson R.S., Tagore K., Haseler L., Jordan M., and Wagner P.D. (1998). Increased  $\text{VO}_2\text{max}$  with a right shifted Hb– $\text{O}_2$  dissociation curve at a constant  $\text{O}_2$  delivery in dog muscle in situ. *J. Appl. Physiol.* 84:995–1002.
- Roca J., Hogan M.C., Story D., Bebout D.E., Haab P., Gonzalez R., Ueno O., and Wagner P.D. (1989). Evidence for tissue diffusion limitation of  $\text{VO}_2\text{max}$  in normal humans. *J. Appl. Physiol.* 67:291–299.
- Sutton J.R., Reeves J.T., Wagner P.D., Groves B.M., Cymerman A., Malconian M.K., Rock P.B., Young P.M., Walter S.D., and Houston C.S. (1988). Operation Everest II: oxygen transport during exercise at extreme simulated altitude. *J. Appl. Physiol.* 64:1309–1321.
- Torrance J., Jacobs P., Restrepo A., Eschbach J., Lenfant C.S., and Finch C.A. (1970). Intraerythrocytic adaptation to anemia. *N. Engl. J. Med.* 283:165–169.
- Valeri L.R. and Fortier N.L. (1969). Red-cell 1,2-diphosphoglycerate and creatine levels in patients with red-cell mass deficits or with cardio-pulmonary insufficiency. *N. Engl. J. Med.* 281:1452–1455.
- Wagner P.D. (1977). Diffusion and chemical reaction in pulmonary gas exchange. *Physiol. Rev.* 57:257–312.
- Wagner P.D. (1996). A theoretical analysis of factors determining  $\text{VO}_2\text{max}$  at sea level and altitude. *Respir. Physiol.* 106:329–343.
- Wagner P.D. (1997). Insensitivity of  $\text{VO}_{2\text{MAX}}$  to hemoglobin— $\text{P}_{50}$  at sea level and altitude. *Respir. Physiol.* 107:205–212.
- Wagner P.D., Laravuso R.B., Goldzimmer E., Naumann P.F., and West J.B. (1975). Distribution of ventilation/perfusion ratios in dogs with normal and abnormal lungs. *J. Appl. Physiol.* 38:1099–1109.
- Wagner P.D., Sutton J.R., Reeves J.T., Cymerman A., Groves B.M., and Malconian M.K. (1987). Operation Everest II: pulmonary gas exchange during a simulated ascent of Mt. Everest. *J. Appl. Physiol.* 63:2348–2359.
- Wagner P.D., and West J.B. (1972). Effects of diffusion impairment on  $\text{O}_2$  and  $\text{CO}_2$  time courses in pulmonary capillaries. *J. Appl. Physiol.* 33:62–71.
- Wallis J.P., Wells A.W., Whitehead S., and Brewster N. (2005). Recovery from post-operative anemia. *Transfusion Med.* 15:413–418.
- West J.B. (1983). American medical research expedition to Everest, 1981. *Himalayan J.* 39:19–25.
- West J.B. (1995). *Respiratory Physiology—The Essentials*, 5th ed. Williams & Wilkins, Baltimore.
- Winslow R.M., Monge C.C., Statham N.J., Gibson C.G., Charache S., Whitttembury J., Moran O., and Berger R.L. (1981). Variability of oxygen affinity of blood: human subjects native to high altitude. *J. Appl. Physiol.* 51:1411–1416.
- Winslow R.M., Samaja M., and West J.B. (1984). Red cell function at extreme altitude on Mount Everest. *J. Appl. Physiol. Respir. Environ. Exerc. Physiol.* 56:109–116.

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